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Abbreviations:

CoA : Coenzyme A
DAG : Diacylglycerol
DGD : DGDG synthase
DGDG : Digalactosyldiacylglycerol
DPG : Diphosphatidylglycerol
ER : Endoplasmic reticulum
MGD : MGDG synthase
MGDG : Monogalactosyldiacylglycerol
LTP : Lipid transfer protein
LysoPC : Lysophosphatidylcholine
PA : Phosphatidate
PAP : Phosphatidate phosphatase
PC : Phosphatidylcholine
PE : Phosphatidylethanolamine
PG : Phosphatidylglycerol
PI : Phosphatidylinositol
PI-TP: Phosphatidylinositol transfer protein
PS : Phosphatidylserine
SQD : SQDG synthase
SQDG : Sulfoquinovosyldiacylglycerol

Abstract

Membranes of plant organelles have specific glycerolipid compositions. Selective distribution of lipids at the levels of subcellular organelles, membrane leaflets and membrane domains reflects a complex and finely tuned lipid homeostasis. Glycerolipid neosynthesis occurs mainly in plastid envelope and endoplasmic reticulum membranes. Since most lipids are not only present in the membranes where they are synthesised, one cannot explain membrane specific lipid distribution by metabolic processes confined in each membrane compartment. In this review, we present our current understanding of glycerolipid trafficking in plant cells. We examine the potential mechanisms involved in lipid transport inside bilayers and from one membrane to another. We survey lipid transfers going through vesicular membrane flow and those dependent on lipid transfer proteins at membrane contact sites. By introducing recently described membrane lipid reorganisation during phosphate deprivation and recent developments issued from mutant analyses, we detail the specific lipid transfers towards or outwards the chloroplast envelope.

Contents

1. Introduction

Plant cell membranes contain a wide range of glycerolipids which are not randomly allocated. A selective distribution at the levels of subcellular organelles, membrane leaflets and membrane domains indicates a complex and finely tuned lipid homeostasis. Additionally, the distribution remains relatively well conserved among tissues and plants species (Table 1). In standard plant culture conditions, on one hand, glycolipids, i.e. sulfolipid and galactolipids, are restricted to plastid membranes and, on the other hand, phospholipids are the main components of extraplastidic membranes. PE is excluded from plastidic membranes, PC is absent from the inner membranes of chloroplasts and DPG is restricted to mitochondria inner membrane. Sterols and glycosphingolipids are present in plant membranes although in much lower amounts than glycerolipids [for reviews see 1, 2]. Like in mammalian cells, sterols and glycosphingolipids are not homogenously distributed among membranes and are notably present in the plasma membrane where they are likely involved in membrane domain organisation.

In plants, phosphate deprivation has been reported to decrease the phospholipid content, consistent with a mobilization of the phosphate reserve, and conversely to increase non-phosphorous membrane lipids such as DGDG and SQDG [3, 4]. Moreover a form of DGDG with specific fatty acids: 16:0 at glycerol sn1 position and 18:2 at sn2 position is especially enhanced [5, 6]. Correlated to the lipid composition changes there is also a strong modification of the lipid distribution between membranes. Upon phosphate deprivation, DGDG, a specific plastid lipid in standard conditions, was found in the plasma membrane [7, 8], a membranous compartment disconnected from plastid membranes, but dynamically connected to the overall endomembrane system. Moreover, several lines of evidence have

shown that large amounts of DGDG are also present in mitochondria upon phosphate deprivation [9].

One cannot explain the membrane specific lipid distribution only by metabolic processes confined in each membrane compartment simply because most lipids are not just present in membranes where they were synthesised. The lipid distribution is usually stable despite the extensive membrane trafficking that occurs between organelles. The maintained specific lipid distribution relies therefore on molecular mechanisms of lipid transport. These mechanisms have been studied mostly in yeast and in mammalian cells [for a review 10] and very few data have been collected in plant models. Besides information from genetics and proteomics analyses, we believe that disturbance of lipid distribution during phosphate deprivation represents a promising tool to unravel these molecular mechanisms in plants. In this review, we discuss recent data describing the potential mechanisms involved in glycerolipid trafficking in plant cells, including recently described membrane lipid reorganization during phosphate deprivation.

2. Membrane glycerolipid diffusion

Membrane lipids are subjected to three kinds of dispersive forces: lateral diffusion, diffusion across membranes (flip-flop) and movement outside the source membrane, possibly reaching other membranes through aqueous phase. Based on the very rapid spontaneous lateral diffusion of lipids within membrane lateral surfaces (0.1 to 1 $\mu m^2.s^{-1}$; [11]), the lipid distribution in vesicular connected organelles should be homogenous. The fact that this is clearly not the case indicates that very dynamic homeostatic mechanisms are involved to maintain the lipid composition of each organelle.

In comparison with lateral diffusion, lipid diffusion between the two leaflets of a membrane bilayer or from one membrane to another is very slow (Figure 1). The spontaneous flip-flop

movement through a lipid bilayer takes less than one second for DAG but it takes hours for lipids with larger polar heads [12].

Lipid diffusion between different membranes is fast for single fatty acid chain lipids that easily partition into the aqueous phase, like lysoPC, or lipids containing two short fatty acid chain (4 to 6 carbons) [12, 13]. By contrast, there is almost no spontaneous transfer of regular diacyl lipids although this trafficking is essential for organelle functioning and cell survival. Vesicular and non-vesicular transport systems have been reported in plants as in other eukaryotic cells. The vesicular pathway supports a complex membrane flow which couples lipid and protein movements while non-vesicular pathways require monomeric exchange of single lipid molecules. Unlike the vesicular pathway, non-vesicular pathways do not involve membrane fusions but rather tight contacts between membranes. A number of enzymes are required to ensure and regulate lipid movements and it is likely that enzymes are also necessary to control lateral and transbilayer movements both at membrane fusion points in vesicular processes and at contact sites in non-vesicular processes.

3. Flip-flop movements

Several types of flip-flop movement exist [for review, see 14]. The spontaneous movement across the bilayer is dependent only on the physical and chemical properties of lipids and membranes. *In vivo*, DAG movement seems mostly driven by diffusive forces whereas proteins are required for significant rates of phospholipid and glycolipid transbilayer movements. Energy-independent flippases catalyse an ATP-independent movement, in which lipids probably move along the polar surface created by these proteins [15]. These flippases can be specific for some lipid classes but they cannot assist the movement of lipids against a physicochemical gradient. They maintain bilayer symmetry by contrast with ATP-dependent

translocases that can accumulate specific lipid classes against equilibrated gradients. ATP-dependent translocases are involved in the preservation of membrane asymmetry [16].

3.1. Energy-independent flippases

To our knowledge, no flippase has been characterized in plant cells although some gene sequences exhibit homologies with flippases from other organisms. The biosynthesis of ER phospholipids is supposed to be localised in the cytosolic leaflet like in other eukaryotic cells [17], therefore half of the newly synthesised lipids has to be transferred to the other leaflet. In yeast and mammalian cells, the ATP-independent flip-flop movement in the ER membrane is ten times faster than in membranes that do not contain proteins. This movement is sensitive to proteases [14] but it is unclear whether it involves a dedicated type of enzymes or the mere presence of proteins. Interestingly, peptides that mimic the α-helices of transmembrane proteins can stimulate phospholipid flip-flop in liposomes [18]. Enhanced flippase-type transfers are also necessary at membrane contact sites where lipids should be transferred from the cytosolic leaflet of the donor membrane to the cytosolic leaflet of the acceptor membrane [19]. It is possible that such flippases are involved in the transfer of DGDG from chloroplasts to mitochondria during phosphate deprivation since this transfer seems related to membrane contact [9 and see below].

The first flippases that have been characterized in eukaryotic cells are the RFT1 protein, a yeast reticulum pyrophosphoryloligosaccharide-dolichol flippase [20], and the scramblase, a red blood cell plasma membrane calcium-dependent flippase [21]. RFT1 transfers dolichol from the reticulum cytosolic leaflet, where it is synthesised, to the lumenal leaflet where it is eventually located [20]. In Arabidopsis, one RFT1 homologue can be retrieved (At5g07630), but is still functionally uncharacterized. In red blood cell membranes, sphingomyelin and most of the PC are located in the plasma membrane outer leaflet whereas PS and a part of the

PE are in the cytosolic leaflet. An increase of calcium influx activates the scramblase, which equilibrates the phospholipid composition between the two plasma membrane leaflets, by catalysing bidirectional transfer of each lipid species. Movement of PS to the outer leaflet can subsequently induce blood coagulation [21]. In Arabidopsis, a scramblase-related gene has a weak but significant similarity (At2g04940) and should be examined in the context of lipid transfers.

3.2. Energy-dependent translocases

In plants, many reports highlight asymmetric distributions of lipids in several membranes. The plant plasma membrane is asymmetric, like in red blood cells, and PS externalisation can induce cell death processes [22]. The above-mentioned scramblase-like plant protein may play a role in affecting the plasma membrane asymmetry. The thylakoids show a marked asymmetry in the transversal distribution of the lipids with an enrichment of the outer leaflet in MGDG and PG while DGDG and SQDG are essentially confined to the inner leaflet [23]. Since these lipids are linked to specific structures of the photosystems, the lipid asymmetry of the thylakoids is likely to reflect the organisation of the photosynthetic machinery [24]. It has also been proposed that the asymmetry is preestablished in the inner envelope membrane due to the topography of lipid synthesising proteins and is transferred to thylakoids by membrane fusion [25]. In the plastid two-membrane envelope, PC is present only in the cytosolic leaflet of the outer membrane [26]. The tonoplast membrane exhibits a slight asymmetry due to a higher amount of PE in the cytosolic leaflet [27]. The biological significance of the asymmetry of plastid envelope and tonoplast membranes is still unknown but is possibly related to signalling or membrane identification mechanism.

Two translocase families are well documented. The first family includes aminophospholipid translocases that belongs to the P-type ATPase superfamily. The first identified enzyme of

this family was DRS2, a yeast translocase [28]. The *drs2* mutant is cold-sensitive and has no asymmetrical accumulation of PS in the inner leaflet of the plasma membrane. Although the initial study was controversial, new data supports the proposal that DRS2 plays a role in the translocation of lipids in the trans-Golgi and endosomal compartments during budding of membrane vesicles [for review, see 14]. In *Arabidopsis*, eleven genes belong to this family (ALA1 to ALA11) (Table 2). ALA1 exhibits the strongest homology to DRS2 and its expression in *drs2* yeast mutant restores cold resistance and internalisation of the plasma membrane PS [29]. However, ALA1 localization and function *in planta* are not characterized. Furthermore, the roles of the other ALA proteins remain speculative.

The second family belongs to the ABC (ATP-binding cassette) protein superfamily that comprises transporters for a whole variety of organic and inorganic compounds. Typically, ABC proteins possess two nucleotide-binding domains and two transmembrane domains. Multi-drug resistance studies in cancerous cells and in yeast first suggested that some ABC transporters participated in the externalisation of plasma membrane lipids and there are now many indications for lipid transport mediated by ABC proteins in cellular membranes [for reviews, see 30, 31]. *Arabidopsis* contains 129 genes belonging to this family [32]. Data on plant ABC transporters indicate that they play a key role in numerous processes necessary for plant development: chlorophyll biosynthesis, iron-sulphur cluster formation, stomata movement and possibly ionic fluxes [for review, see 33]. Recent reports have pointed out their role in plant lipid transport (Table 2). A potential transporter of plastid fatty acids, identified in the *Arabidopsis* genome (At1g54350), belongs to this family [34]. The major site of *de novo* synthesis of fatty acids in plant cells is the plastid stroma. Fatty acids are produced predominantly as C18:1-ACP and C16:0-ACP and are metabolic substrates for numerous pathways such as membrane glycerolipid biosyntheses in the plastid envelope as well as in the

ER [for a review see 2]. An acyl-ACP thioesterase, localized in the inner envelope membrane, can hydrolyze acyl-ACP and release free fatty acids. Free fatty acids released from the inner envelope are channelled to the outer envelope, possibly via the At1g54350 ABC transporter and reactivated to acyl-CoA in the outer envelope [35].

Xu *et al.* identified another chloroplastic ABC type protein (TGD1) (At1g19800) that is likely dedicated to the import of phosphatidate from the ER to the chloroplast for the synthesis of eukaryotic galactoglycerolipids [36, 37]. The so-called prokaryotic and eukaryotic types of galactoglycerolipid structures exist in plants [for a review see 2]. Both types are generated in the chloroplast envelope by galactosylation of DAG molecular species of distinct origins. Whereas prokaryotic DAG is fully synthesized in plastids, eukaryotic DAG originates from PC formed in the ER. Studies with labelled lipid precursors indicated that PC provides its eukaryotic DAG backbone to plastid glycerolipids [38, 39]. This transfer requires desaturated PC since the *fad2 Arabidopsis* mutant, deficient in the desaturation of C18:1 localised in ER, contains a smaller eukaryotic/prokaryotic monogalactosyldiacylglycerol (MGDG) ratio than wild type plants [40]. Although PC is present in the outer membrane of the plastid envelope, the only known site of PC *de novo* biosynthesis is localised in the ER. A direct import of PC from ER, or an import of intermediates derived from endoplasmic reticulum PC, possibly DAG or phosphatidate, is therefore required for the synthesis of eukaryotic galactolipids (Figure 2). It is still unclear which lipid is transported. Some clues have been deduced from the in-depth examination of lipids of plant cell suspensions after transferring the cells into a phosphate-deprived medium [6]. Since phosphate starvation induced a high increase in the synthesis of eukaryotic DGDG (notably the above-mentioned 16:0-18:2 molecular species), the exposure to phosphate starvation conditions was therefore a way to analyze the passage of the DAG-backbone from the ER PC to the plastid galactolipids. Phosphate starvation induced

an increase of DAG with an identical fatty acid composition as PC. Since DAG was not detected in plastid membranes and a phosphatidate pool was not detected in the cells, Jouhet *et al.* proposed that DAG is the molecule transported from ER to chloroplast. Several phospholipases C specifically activated during Pi deprivation are probably involved in the formation of this DAG pool [41, 42]. The transferred DAG is presumably galactosylated into MGDG and DGDG through the actions of the enzymes MGD2 (or MGD3) and DGD1 (or DGD2) induced by phosphate deprivation and present in the outer envelope membrane.

However, the existence of a phospholipase D (PLDζ2) activated by phosphate deprivation [42, 43], the accumulation of phosphatidate in the tgd1 mutant and the localization of TGD1 in the chloroplast inner envelope membrane [37] suggest that the transported molecule could be phosphatidate. To understand this latter scheme, the precise localization of the phosphatidic acid phosphatase (PAP) involved in the transformation of eukaryotic phosphatidate into DAG is crucial since part of the eukaryotic DAG is galactosylated by MGD1, the constitutive MGDG synthase that is present in the inner envelope membrane. In some plants such as spinach, PAP has been detected in the inner envelope membrane and this was considered to be dedicated to the formation of prokaryotic DAG [for a review see 2]. Therefore, either this PAP is also involved in the formation of eukaryotic DAG or a complementary transport of eukaryotic DAG is necessary. Moreover, in some other plants such as pea, even though MGDG synthase is present in the inner envelope membrane, PAP activity has never been detected in the envelope, possibly related to the fact that no prokaryotic DAG is formed in these plants. Therefore, at least in plants such as pea, transport of eukaryotic DAG up to the inner envelope membrane is necessary to ensure MGDG synthesis and thylakoid development. Altogether, it is likely that in all plants eukaryotic DAG is transported from the ER to the inner envelope membrane. Recent findings from the tgd1

mutant analysis indicate that eukaryotic phosphatidate is also transported to the inner envelope membrane where it plays a role in enhancing of MGDG synthesis (Figure 2).

4. Vesicular lipid transfer

4.1. Secretory pathway

Since most phospholipids, sterol, sphingolipid and many proteins are synthesized in the ER, this compartment can be considered as a cell central metabolic organelle in relation with other compartments. During interphase, the ER is in direct connection with the nucleus. On electron micrographs, the ER and nucleus membranes appear as connected, although a lumen constriction is observed at the junction area [44]. With or without Golgi participation, the ER is involved in protein and lipid supply for the vacuolar and plasma membranes by vesicles moving along actin filaments [45].

The general mechanism of vesicular transfer is carried out in several steps [for reviews see, 46, 47]. Briefly, small type Ras GTPases, coupled to GTP exchange factors, support the recruiting of protein complexes and their anchoring to the donor membrane. Coat proteins, like adaptatin or clathrin, can participate with these complexes. Then, vesicles extrude from the donor membrane (following mechanisms that remain to be fully characterized), and move away using the actin cytoskeleton and myosin. Dynamin, a large GTPase is involved in the scission of nascent vesicles from the parent membrane. Anchoring and fusion to the target membrane are mediated by SNARE (Soluble N-ethylmaleimide-sensitive factor Attachment protein REceptors) proteins, located both on the vesicles (v-SNARE) and on the target membrane (t-SNARE), and small GTPases.

This process has been intensively studied at the level of protein trafficking and, although vesicles obviously transfer lipid material, very little data on lipids exist. In the following paragraphs, we present current information on the molecular machineries involved in

vesicular trafficking in plants, specifically in endomembranes (Figure 3) and from chloroplast envelope to thylakoids. This is then developed in relation to specific lipid transfers.

4.1.1. Reticulum and Golgi exchanges

These exchanges are mediated by transport vesicles that bud from the donor membrane due to the assembly of a cytosolic coat protein known as coatomer protein I or II. Vesicles containing these proteins are called respectively COPI and COPII. The ER to Golgi pathway transfers phospholipids, sterols, sphingolipids and proteins. In mammalian and yeast, this transfer is mediated by COPII-type coated vesicles. In plants, the Golgi apparatus is close to the ER but, although proteins exhibiting substantial homology with partners of the COPII complex are known, it was never possible to visualize any COPII-type vesicles. The ER to Golgi transfer does not seem to imply a vesicular stage [48]. It probably works with the COPII machinery but a COPII independent pathway is also possible [49]. Surprisingly, the transfer from ER to Golgi is independent of actin or microtubules [50]. The long-standing contact between the reticulum and the Golgi is apparently sufficient to support the transfer.

Several studies have shown the existence of a Golgi to ER pathway in plants presumably working through COPI vesicles [48]. There is no doubt that the COPI machinery exists in plants and it is likely that COPI and COPII pathways are interdependent due to the necessity for recycling the regulatory machinery of the secretory pathway. The exact role of COPI vesicles in the Golgi to ER transfer has been a matter of controversy mostly because much of the information comes from use of the secretory pathway inhibitor BFA, which affects also the ER to Golgi pathway. No data is available about the importance of this COPI and COPII trafficking on the lipid distribution in the cell.

4.1.2. Transfer to the vacuoles

In plants, two types of vacuoles exist. On one hand, the storage vacuole is present in the storage tissues, like cotyledons and endosperm. It is characterised by a neutral pH and by the

presence of α-TIP (tonoplast integral protein) and storage proteins, like prolamins and globulins. On the other hand, the lytic vacuole of vegetative tissues is characterised by an acidic pH and presence of γ-TIP and lumenal proteins. Theses two vacuoles have different protein-sorting mechanisms [44], and little is known about their lipid sorting.

Although the storage vacuole lipid composition is not yet characterised, different mechanisms of transfers are identified depending on tissues and plant species. Two types of vesicles are possibly present: the first type originating from the ER, corresponds to the so-called "precursor-accumulating compartment" that contains prolamins; the other type (deriving from the Golgi), corresponds to dense vesicles that contain globulins [51]. Altogether, proteins destined for the storage vacuole have an addressing domain in their C-terminal and their transfer involves the activation by PI-4P phosphoinositide since this trafficking is sensitive to Wortmaninn, a PI-kinase inhibitor [52].

The lytic vacuole can be compared to animal lysosomes. Lipids from clathrin-coated vesicles are transferred from the Golgi to a prevacuolar compartment, with the intervention of the actin cytoskeleton and dynamin [45]. Transfer between the prevacuolar compartment and the lytic vacuole is not yet characterised. PI-3P is involved in the vesicular transfer from the Golgi to the lytic vacuole. In animals and yeast, a protein called EEA (Early Endosome Antigen) binds PI-3P with the participation of RabF GTPase and drives to the endosome fusion. The overexpression of the EEA PI-3P binding domain in *Arabidopsis* showed that this domain was sorted gradually from the Golgi to the lytic vacuole *via* the prevacuolar compartment. By competition mechanism, this overexpression inhibited the trafficking of vacuole proteins while trapping free PI-3P [53]. The plant ADL6 dynamin, that binds adaptatin and actin, has a PI-3P specific PH domain (phosphoinositide recognition domain) and its mutation abolished the trafficking of vacuole proteins. ADL6 is therefore an important protein that connects PI-

3P local concentration and sorting to the vacuole [54]. However, nothing is known about the lipid composition and the lipid selectivity of all these vesicles.

4.1.3. Transfer to the plasma membrane

The plant cell plasma membrane is not homogenous and domains are detected. A domain is a membrane area, organised or not, whose lipid and/or protein composition differs from the bulk membrane composition. For example, rafts are membrane domains that resist detergent solubilization due to a special lipid/protein local composition. Lipid rafts were recently characterised in Tobacco and *Arabidopsis* [55, 56]. Rafts are enriched in sterols and sphingolipids and recruit a pool of specific proteins. Some other plant plasma membrane domains have been characterized by their divergent protein composition and their position in the cell: for example, in vascular cells, the auxin efflux transporter, PIN1, is localised in the basal pole whereas the auxin influx transporter is found at the apical pole [46].

The plasma membrane derives from uncharacterized Golgi vesicles. Lipid trafficking from the Golgi to the plasma membrane occurs partly through these transport vesicles. The nature of the vesicles is not yet identified although the following data support their existence. It has been recently shown by live imaging of animal cells that transport vesicles from the Golgi directly fuse with the plasma membrane [57]. Consistently, in plant, monensin, a secretory pathway inhibitor, was shown to cause accumulation of part of the plasma membrane lipids in the Golgi [1]. This pathway is actin dependent [45]. The addressing of proteins to the plasma membrane seems to be controlled by the size and number of their hydrophobic domains [58]. Thus, preferential interactions between lipids and transmembrane proteins were proposed to manage protein sorting in the Golgi. In the future, it will be important to define the precise origins and fusion sites of vesicles in order to understand the general formation of the plasma membrane and the genesis of particular microdomains. Concerning rafts, one can suggest that,

according to animal cell data, domains may be organized in the Golgi and subsequently exported to the plasma membrane [59].

PIP_2 plays a role in the transport to the plasma membrane [60]. A PI transfer protein (PI-TP) was identified in *Arabidopsis* and was shown to be a SEC14 homolog allowing the complementation of a yeast *sec14* mutant. In yeast, the inactivation of SEC14 impedes protein secretion at the Golgi level. Interestingly, mutations in genes involved in the nucleotidic pathway for PC biosynthesis abolish the effect of the *sec14* mutation. In fact, SEC14 binds both PC and PI and is a phosphocholine:CTP cytidylyltransferase inhibitor. It has been proposed that SEC14 was a "sensor" of the plasma membrane lipid composition and could inhibit PC biosynthesis when PC is abundant. This hypothetical role has not been confirmed *in planta* [61]. Another PI-TP, AtSfh1p, belonging to the SEC14-nodulin family, was identified in *Arabidopsis*. AtSfh1p is likely a regulator of the intracellular trafficking in root hair by moving PIP_2 to the areas of the plasma membrane where vesicles fuse [62].

By using monensin, Moreau *et al.* [1] tried to determine which lipid classes were transferred to plant plasma membrane *via* the vesicular pathway. When monensin was added, these authors observed in the plasma membrane the absence of PS, whereas the PI content was not affected, and PE and PC amounts were half reduced. Based on these results, they concluded that PS was exclusively transferred to the plasma membrane by vesicular pathway, PE and PC were partly transferred by the vesicular pathway and PI was transferred by another process. However Vincent *et al.* [63] observed that PS could also be synthesised directly in the plasma membrane by polar head exchange. The balance between PS from Golgi vesicles and PS generated by plasma membrane polar head exchange is unknown.

4.1.4. Endocytosis and exocytosis

Plant endocytosis was questioned for long due to the turgid pressure and cell wall rigidity that were supposed to prevent its existence. It is now well established that brefeldin A, an

exocytosis inhibitor, induces the internalisation of plasma membrane proteins, like the auxin-carrier proteins AUX1 and PIN1, in a compartment called the endosome [45]. The distinction between the endosome and prevacuolar compartments is not clear [64] because the fate of internalised proteins divides in two parts: a part is recycled to the plasma membrane and the other destined for the lytic vacuole.

Plant endocytosis implies at least two pathways: one is catalysed by clathrin-coated vesicles that are transported by the actin cytoskeleton and moved by myosin VIII [45]; another one, like the PIN endocytosis, depends on presence of sterols and of rafts [65]. In plants, no receptor for addressing sequences to clathrin-coated vesicles has been identified despite pointed homologies with some yeast receptors in the *Arabidopsis* genome [64]. However, two subunits of the AP2 adaptatin complex, αC-adaptatin and AP180, were characterised in *Arabidopsis*. AP180 catalyses clathrin assembly and αC-adaptatin binds to AP180 and to a dynamin like protein, probably ADL1 [66].

The transfer from endosome/prevacuolar compartment to lytic vacuole is not characterized besides a PI-3P control [46]. In contrast, the mechanism of vesicle recycling to plasma membrane is partially characterized. The recycling machinery is actin-dependent and involves Arf1, GNOM and PIN since brefeldin A, an exocytosis inhibitor, aborts the recycling of the PIN auxin efflux facilitators to plasma membrane by inhibiting GNOM activity, *i.e.* GDP-GTP exchange on Arf1, a small GTPase. This pathway is involved in the transfer of other proteins than PIN such as AUX1 and some ATPases.

To conclude, very few studies concern glycerolipid trafficking through the secretory pathway. Altogether data indicate the following general features: a) ER-Golgi trafficking has no glycerolipid specificity whereas b) there is some selectivity concerning trafficking to the plasma membrane from the Golgi apparatus. In this case, PS is mainly transported by vesicles,

PE and PC are partially transferred by this route and PI is moved by another method (independent of the secretory pathway). Moreover, PI has an additional role in the regulation of trafficking. Different phosphorylated forms of PI favour sorting of vesicles to distinct compartments: PI-4P to storage vacuole, PI-3P to lytic vacuole and PIP_2 to plasma membrane.

4.2. Transfer from the plastid envelope to the thylakoids

Glycerolipids i.e. MGDG, DGDG, PG and sulfolipid, synthesised in the plastid envelope [for review see 2] are selectively transported from the envelope inner membrane to the thylakoids [67, 25]. Our knowledge about this lipid transfer mechanism is very imprecise but there are some indications supporting vesicular trafficking. Vesicle budding from the plastid envelope inner membrane was observed by electronic microscopy [68, 69]. An *Arabidopsis thaliana* thylakoid formation 1 (Thf 1) gene product was shown to control a step required for the organization of vesicles derived from the envelope inner membrane into mature thylakoid stacks [70]. Bioinformatic studies suggest that a system similar to the COPII vesicular pathway is present in plastids [71]. The plastid vesicular pathway is dependent on ATP and stromal proteins [72, 73]. Some stromal proteins involved in this trafficking have been identified: a NSF homolog protein [74], a dynamin-like protein [75] and a vesicle inducing protein (VIPP1) [76]. In *vipp1* (a VIPP -Vesicle Inducing Protein in Plastid 1-deleted mutant), thylakoid membrane formation and chloroplast vesicle transport are abolished, indicating that VIPP1 is essential for thylakoid maintenance by a vesicular pathway. Recent data demonstrate that VIPP1 organizes in a high molecular mass complex closely associated with the inner envelope membrane and suggest that the C-terminus of the protein protrudes from the complex into the stroma of chloroplasts possibly for interaction with some other proteins [77]. Accordingly, soluble VIPP1 interacts with a HSP70B/CDJ2 chaperone pair [78]. By analogy with the action of the auxilin/Hsc70 chaperone pair with clathrin on clathrin-coated

vesicles, HSP70B/CDJ2 might disassemble and/or assemble VIPP1 oligomers to recycle the system for another turn of vesicle formation/transport [78].

5. Transfer by membrane contact

Whereas vesicular pathways are involved in the lipid provisioning for thylakoids and organelles of the secretory pathway, this process does not provide every necessary lipid for the plasma membrane. In addition, the secretory pathway is apparently not involved in the important lipid transfer from ER to plastids and from ER to mitochondria since no isolated vesicle has been clearly detected between ER and these semi autonomous organelles. Another type of lipid transfer has been proposed involving membrane contact between two organelles [79]. By contrast with vesicular trafficking, transfer by contact does not involve a transfer of a portion of a bilayer as such but a selective transfer of some components of the membrane. In plants, some indications of a lipid transfer by membrane contact between plastid envelope and mitochondria were recently reported [9].

5.1. Membrane contact sites

In a topological plan at the cell scale, ER has many specialised sub domains that interact with other organelles such as mitochondria, vacuole, plasma membrane or plastids [44] (Figure 4). At a membrane contact site, membranes are typically separated by less than 10 nm [10]. The best-characterized membrane contact site was described in yeast between ER and mitochondria [19] during a study of the mechanisms for PE biosynthesis in mitochondria [80]. PE biosynthesis in mitochondria occurs by decarboxylation of PS and is dependent on PS supply by ER [79]. Specific ER domains called MAM (Mitochondria Associated Membranes) are enriched in PS synthase, supporting PS supply to mitochondria. The PS transfer from ER to the mitochondria outer membrane is not completely characterized. Data indicate ATP dependence of PS transfer, Ca2+ and Mg2+ stimulation, participation of a S100B protein

(Ca2+-modulated protein of the EF-hand type) that binds calcium and can interact with annexin, and existence of a regulatory system involving a SCF (Skp1/Cul1/F-box protein) ubiquitin ligase [19]. PS is finally transported to the mitochondria inner membrane by an unknown mechanism, maybe going through contact sites between the inner and outer membranes of mitochondria [81]. PS is decarboxylated in the mitochondria inner membrane to form PE before a retro-transfer to ER probably going also through MAM [79]. Plants are known to synthesize ethanolamine moieties of PE mainly by decarboxylation of free serine, but there is also some evidence for PS decarboxylation, with both mitochondrial and extramitochondrial PS decarboxylases [82, 83]. It is very likely that, in plants, the same kind of PS transfer through MAM occurs for synthesis of some part of the mitochondrial PE. In addition, MAM are also possibly involved in transfer of other mitochondrial phospholipids synthesized in ER such as PC and PE.

Concerning transfer from ER to plasma membranes, it is believed, based on yeast studies, that PE is transferred by a similar kind of system involving PAM (Plasma membrane Associated Membrane) domains [10]. In plant, PAM-plasma membrane interactions contain actin cytoskeleton elements and are sensitive to cell wall digestion [84].

Finally, similar interactions by membrane contacts were observed between ER and vacuoles. The ER anchoring to vacuole or to plasma membrane may serve as semi-immobile platforms to which actin filaments attach to drive endoplasmic streaming [44]. These contact sites possibly participate in the monensin insensitive transfer of PE, PC and PI, previously described, with the involvement of LTPs (see below).

By analogy with MAM, PLAM (PLastid Associated Membrane) are supposed to be present at the periphery of the chloroplast [85]. PLAM could contribute to the transfer of eukaryotic DAG backbone from ER to plastids as described above. They could also facilitate the transfer

of PC from the ER to the outer membrane of the chloroplast envelope (Figure 2). Actually, partial hydrolysis of PC to LysoPC in the ER was proposed to favour PC transfer between ER and chloroplast since amphiphilic lysoPC can move easily through the cytosol [86]. In support to this hypothesis, a lysoPC acyltransferase activity was detected in the chloroplast envelope [86].

Plant organelles are mobile inside the cell. Therefore, they can create membrane contact sites adapted to physiological need. Each organelle has its own mechanism for location within the cell and all organelle positions are coordinated in order to let each organelle fulfil its function [87]. These movements are mainly actin-dependent in vegetative cells and this mobility supports formation of organelle contact sites.

Plastids produce also very dynamic tubular structures called stromules that are able to interact with other plastids and even with other type of organelles (Figure 4). Stromules are highly dynamic structures involving both membranes of the plastid envelope and containing stroma [for review, see 88]. In different tissues of tobacco and *Arabidopsis*, stromules interact with the nucleus, the plasma membrane and mitochondria. They possibly support transfers such as DAG, PC or DGDG transfers, although this role of stromules has never been demonstrated [89, 90]. Other plastid envelope structures have been visualised to resemble myelin. These structures were proposed to favour transfer of substances from plastid to vacuoles [91].

5.2. Lipid transfer protein or LTP

Lipid-transfer proteins that shuttle across cytosolic gaps mediate the trafficking of particular lipids between organelles [10]. These proteins have to target both donor and acceptor membranes and, indeed, some LTP have been localized at membrane contact sites where two membranes were in close proximity [92]. For instance, LTPs of the Osh multigenic family (Osh1, 2 and 3) were localised at reticulum contact sites in yeast. Osh LTPs belong to the

oxysterol binding protein (OSBP) family and are presumably involved in the movement of sterol among cellular compartments, as was recently demonstrated for Osh4p [93]. In *Arabidopsis*, twelve homologs of OSBP were identified but none has been studied yet (Table 2). Some LTPs have two targeting domains, one for the lipid donor membrane and the other for the lipid receptor membrane. Consequently, LTPs can either move from one membrane to another or bind to both membranes at the same time [10]. Holthuis and Levine pointed that simultaneous binding to both membranes should promote lipid transport. Since the absence of membrane fusion is a characteristic of membrane contact sites, one can question which proteins are involved for the stabilisation of the membrane contact sites and whether LTP may contribute to such stabilization.

To date, only one protein "bridge" between two organelles has been characterized between the nucleus and the vacuole in yeast [94]. This "bridge" contains one nuclear envelope outer membrane protein (Nvj1p) that docks to a vacuole surface protein (Vac8p). This binding supports the formation of membrane contact sites between these two organelles without membrane fusion.

Other LTP families were characterised without any information concerning their possible localization to membrane contact sites. StART (Steroidogenic-Acute-Response protein related lipid Transfer protein) proteins have a conserved domain around 200 amino acids involved in lipid or sterol binding. A protein of the StART family, CERT (CEramide Related Transfer protein), was recently characterised in mammalian cells [95]. CERT has a reticulum and a Golgi binding domain and transfers ceramide from the reticulum to the Golgi.

Thirty-five proteins containing StART domains were identified in *Arabidopsis*. No CERT close homolog was found in plants, but six proteins are homologues to StARD2, a PC-binding protein [96]. The *in silico* studies of StARD2 homolog genes suggest that their products are

addressed either to mitochondria, or to plastids or to the secretory pathway (Table 2). These proteins are possible candidates for the vesicular independent trafficking of PC, the eukaryotic DAG backbone or of the minus Pi-induced mitochondrial DGDG.

The GLTP (GLycolipid Transfer Protein) family includes proteins involved mainly in glycosphingolipid transfer; this family contains four genes in the *Arabidopsis* genome (Table 2). The *Arabidopsis* ACD11 protein is involved in the cell death mechanism and, by contrast with the mammalian protein, it transfers only sphingosine [97]. No evidence was found of the GLTP involvement in galactolipid transfer in plants but because of some similarities of galactocerebroside to the glycosylglycerides, this hypothesis remains possible.

In plants, some PI-TPs are also present. SEC14 and AtSfh1, previously described, are some examples. There are probably other PI-TP types although not yet known [62] (Table 2).

Finally, a SCP2 (Sterol Carrier Protein 2) was identified in *Arabidopsis*, transferring sterols as well as phospholipids (Table 2). SCP2 is involved in the lipid transfer to the peroxysome for the lipid catabolism [98].

In plants, a large family of small soluble LTPs has been very well characterised genetically and structurally [for reviews, see 99, 100]. The proteins transfer lipids *in vitro* between two membranes and have no specificity for their lipid substrate. Sixty-seven genes were identified in *Arabidopsis* (Table 2), among which most of them have a secretory peptide, indicating that these proteins are excreted. The biological role of these proteins is unknown. They seem to be involved in embryogenesis, cell wall formation and pathogen resistance [101].

6. Conclusion

Although requiring fatty acid delivery from the chloroplast, the plant ER is considered as a lipid autonomous organelle because most of the phospholipid biosynthesis occurs there. The nucleus and the Golgi are connected to the reticulum without any glycerolipid selection either

by membrane continuity or by dynamic vesicle exchanges. Vacuoles have vesicular connection to the ER and the plasma membrane *via* the prevacuolar compartment but nothing is known about the lipid delivery. More information is available concerning the lipids in the plasma membrane. In particular, PS and a part of PE and PC are known to derive from the Golgi by vesicular pathway. PI and part of PE and PC may come from the ER through PAM and the plasma membrane DGDG, induced upon phosphate deprivation, could derive from plastids either *via* direct contact with plastids possibly involving stromules, or *via* the ER.

Semi-autonomous organelles such as mitochondria and plastids are not connected to the ER via vesicles. Lipid supply to mitochondria seems to occur exclusively through membrane contact sites. Besides the DPG that is synthesised *de novo* in mitochondria, phospholipids are transported from ER *via* MAM and, under phosphate deprivation, DGDG arrives through the plastid envelope-mitochondria contact sites. Plastids are relatively independent for their lipid synthesis due to the several lipid synthetic activities present in the envelope, but they need a supply of a diacylglycerol source from the ER for formation of their eukaryotic glycerolipids. This transfer is likely achieved via PLAM through a direct transfer of DAG molecules. However phosphatidate derived from reticulum PC plays also some role in plastid envelope galactolipid synthesis and the ABC protein TGD1 contributes to its transport to the inner envelope membrane. Contact sites between the two envelope membranes may be involved in the transfer since there is an intricate contribution of enzymatic activities of either the outer envelope membrane or the inner envelope membrane (for instance, for the synthesis of DGDG). In addition, plastids cannot achieve PC synthesis and need to import PC or LysoPC from the ER. Inside chloroplasts, MGDG, DGDG, PG and sulfolipid are transferred from the inner envelope membrane to the thylakoids by a vesicular transport that involves the VIPP1 protein.

Altogether, the building of each type of membranes in plant cells requires an intricate relationship between lipid synthesis and lipid transfer. Nothing is known whether the final organisation of membranes is dependent or not on an original membrane pattern. Recent progress concerns lipid transfers but the molecular mechanisms of the transfer are still very elusive. Although some proteins have been identified, many candidates for lipid transfer proteins remain to be identified. One expects that analysis of gene expression under conditions such as phosphate deprivation which can affect lipid distribution should provide new candidate proteins for these transfers and their regulation. Since all lipid trafficking occurs in highly dynamic cells with mobile organelles, it is likely that cytoskeleton proteins and molecular motors are essential and closely related to the transport. An important point will be to understand how the lipid to be transferred are selected and when they are selected related to the transfer. Organisation of lipid domains in membranes is likely to be important but selection is expected to be also dependent on affinity of lipid transfer proteins for specific lipids. Finally, dissection of these mechanisms of transfer will be an important challenge for the future.

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Table 1: Lipid composition of plant cell membranes according to [1]. Endomembrane analyses are derived from [102], [103], and [104]. Plastid and mitochondria membrane analyses were done respectively by [105] and [106].

Membranes	PC	PE	PG	PI	PS	DPG	MGDG	DGDG	SQDG	Sterols	Glyco-sphingolipides
Endomembranes											
Reticulum +Golgi	43-48	23-26	6	6	3					4-15	
Tonoplast	15-28	15-28	2	5-9	2					14-43	12-17
Plasma membrane	8-36	9-32	1-5	1-6	1-10					5-60	6-30
Chloroplasts											
Outer membrane	32		10	5			17	30	6		
Inner membrane			9	1			55	30	5		
Thylakoids			7	1			58	27	7		
Mitochondria											
Outer membrane	52	22	3	10						13	
Inner membrane	37	33	2	4		11				13	

Table 2: *Arabidopsis thaliana* genes potentially involved in lipid trafficking. Protein localization determined by TargetP [107] is indicated by P letter for plastid, M for mitochondria and S for secretory peptide presence. Protein localizations reported between brackets were obtained by biochemical experiments [62, 98]. TM: transmembrane domain number.

LTP	Bound lipid	*Arabidopsis* locus	Protein	Evidence	TM	Localization
Flippase						
RFT1	?	At5g07630		Sequence similarity	9	-
scramblase	?	At2g04940		Sequence similarity	0	M
P-type ATPase						
Aminophospholipid translocase	**PS,** PE	At5g04930	ALA1	Functional complementation	10	-
Aminophospholipid translocase	?	At5g44240	ALA2	Sequence similarity	9	-
Aminophospholipid translocase	?	At1g59820	ALA3	Sequence similarity	8	M
Aminophospholipid translocase	?	At1g17500	ALA4	Sequence similarity	10	M
Aminophospholipid translocase	?	At1g72700	ALA5	Sequence similarity	10	M
Aminophospholipid translocase	?	At1g54280	ALA6	Sequence similarity	10	M
Aminophospholipid translocase	?	At3g13900	ALA7	Sequence similarity	10	M
Aminophospholipid translocase	?	At3g27870	ALA8	Sequence similarity	10	M
Aminophospholipid translocase	?	At1g68710	ALA9	Sequence similarity	10	M
Aminophospholipid translocase	?	At3g25610	ALA10	Sequence similarity	10	M
Aminophospholipid translocase	?	At1g13210	ALA11	Sequence similarity	10	M
ABC transporter supposed to be involved in lipid translocation						
ABC acyl transporter	?	At1g54350		Sequence similarity	5	P
ABC transporter	?	At1g19800	TGD1	EMS mutant characterization	6	P
Oxysterol binding protein						
Oxysterol binding protein	?	At1g13170	0sh3	Sequence similarity	0	P
Oxysterol binding protein	?	At2g31020		Sequence similarity	0	P
Oxysterol binding protein	?	At2g31030		Sequence similarity	0	-
Oxysterol binding protein	?	At3g09300		Sequence similarity	0	P
Oxysterol binding protein	?	At4g08180	OSBP	Sequence similarity	0	-
Oxysterol binding protein	?	At4g12460	Osh1	Sequence similarity	0	-
Oxysterol binding protein	?	At4g22540	Osh2	Sequence similarity	0	-
Oxysterol binding protein	?	At4g25850		Sequence similarity	0	-
Oxysterol binding protein	?	At4g25860		Sequence similarity	0	-
Oxysterol binding protein	?	At5g02100		Sequence similarity	0	P
Oxysterol binding protein	?	At5g57240	KES1	Sequence similarity	0	-

LTP	Bound lipid	*Arabidopsis* locus	Protein	Evidence	TM	Localization
Oxysterol binding protein	?	At5g59240		Sequence similarity	0	-
StARD2 homolog proteins						
PC transfer protein	PC	At1g55960		Sequence similarity	1	P
PC transfer protein	PC	At1g64720	CP5	Sequence similarity	1	S
PC transfer protein	PC	At3g13062		Sequence similarity	1	M
PC transfer protein	PC	At3g23080		Sequence similarity	1	-
PC transfer protein	PC	At4g14500		Sequence similarity	2	-
PC transfer protein	PC	At5g54170		Sequence similarity	2	S
CERT	ceramide	?				
Glycolipid transfer protein						
GLTP	sphingosine	At2g34690	ACD11	T-DNA mutant characterization, functional complementation and *in vitro* expression	0	-
GLTP	?	At4g39670		Sequence similarity	0	-
GLTP	?	At2g33470		Sequence similarity	0	-
GLTP	?	At3g21260		Sequence similarity	0	-
PITP						
Type SEC14	PI/PC	At1g55840	SEC14	Functional complementation	0	-
Type SEC14	PI	At4g34580	AtSfh1/ COW1	T-DNA mutant characterization and functional complementation	1	- (Golgi)
Sterol carrier protein						
SCP	PL, stérol	At5g42890	SCP2	*In vitro* expression	0	- (Perox)
Non specific lipid transfer protein						
Lipid Transfer Protein type 1	-	At2g38540	LTP1	*In vitro* expression	0	S
Lipid Transfer Protein type 1	-	At2g38530	LTP2	*In vitro* expression	0	S
Lipid Transfer Protein type 1	-	At5g59320	LTP3	Sequence similarity	0	S
Lipid Transfer Protein type 1	-	At5g59310	LTP4	Sequence similarity	1	S
Lipid Transfer Protein type 1	-	At3g51600	LTP5	Sequence similarity	0	S
Lipid Transfer Protein type 1	-	At3g08770	LTP6	Sequence similarity	0	S
Lipid Transfer Protein type 1	-	At2g18370	LTP7	Sequence similarity	0	S
Lipid Transfer Protein type 1	-	At2g15050	LTP8	Sequence similarity	0	S
Lipid Transfer Protein type 1	-	At2g15325	LTP9	Sequence similarity	1	S
Lipid Transfer Protein type 1	-	At5g01870	LTP10	Sequence similarity	0	S
Lipid Transfer Protein type 1	-	At4g33555	LTP11	Sequence similarity	0	S
Lipid Transfer Protein type 1	-	At3g51590	LTP12	Sequence similarity	0	S
Lipid Transfer Protein type 1	-	At4g08530	LTP15	Sequence similarity	0	-
Lipid Transfer Protein type 2	-	At1g48750		Sequence similarity	1	S
Lipid Transfer Protein type 2	-	At1g66850		Sequence similarity	0	S
Lipid Transfer Protein type 2	-	At1g73780		Sequence similarity	0	S
Lipid Transfer Protein type 2	-	At3g18280		Sequence similarity	1	S
Lipid Transfer Protein type 2	-	At3g57310		Sequence similarity	0	S
Lipid Transfer Protein type 2	-	At5g38160		Sequence similarity	0	S
Lipid Transfer Protein type 2	-	At5g38170		Sequence similarity	1	S
Lipid Transfer Protein type 2	-	At5g38180		Sequence similarity	1	S
Lipid Transfer Protein type 3	-	At1g32280		Sequence similarity	0	S
Lipid Transfer Protein type 3	-	At3g07450		Sequence similarity	0	S
Lipid Transfer Protein type 3	-	At3g52130		Sequence similarity	1	S
Lipid Transfer Protein type 3	-	At4g30880		Sequence similarity	0	S
Lipid Transfer Protein type 3	-	At4g33550		Sequence similarity	0	S
Lipid Transfer Protein type 3	-	At5g07230	A9	Sequence similarity	0	S
Lipid Transfer Protein type 3	-	At5g48485		Sequence similarity	1	S
Lipid Transfer Protein type 3	-	At5g48490		Sequence similarity	0	S
Lipid Transfer Protein type 3	-	At5g52160		Sequence similarity	1	S
Lipid Transfer Protein type 3	-	At5g55410		Sequence similarity	0	S
Lipid Transfer Protein type 3	-	At5g55450		Sequence similarity	0	S
Lipid Transfer Protein type 3	-	At5g55460		Sequence similarity	0	S
Lipid Transfer Protein type 3	-	At5g56480		Sequence similarity	0	S
Lipid Transfer Protein type 3	-	At5g62080		Sequence similarity	0	S
Lipid Transfer Protein type 4	-	At3g53980		Sequence similarity	0	S
Lipid Transfer Protein type 4	-	At5g05960		Sequence similarity	0	S
Lipid Transfer Protein type 5	-	At1g18280		Sequence similarity	0	S
Lipid Transfer Protein type 5	-	At1g27950		Sequence similarity	2	S
Lipid Transfer Protein type 5	-	At1g36150		Sequence similarity	1	S
Lipid Transfer Protein type 5	-	At1g55260		Sequence similarity	0	S
Lipid Transfer Protein type 5	-	At1g62790		Sequence similarity	2	S
Lipid Transfer Protein type 5	-	At1g70240		Sequence similarity	0	
Lipid Transfer Protein type 5	-	At1g73550		Sequence similarity	1	S
Lipid Transfer Protein type 5	-	At1g73560		Sequence similarity	0	S
Lipid Transfer Protein type 5	-	At1g73890		Sequence similarity	1	S
Lipid Transfer Protein type 5	-	At2g13820		Sequence similarity	0	S

LTP	Bound lipid	*Arabidopsis* locus	Protein	Evidence	TM	Localization
Lipid Transfer Protein type 5	-	At2g27130		Sequence similarity	1	S
Lipid Transfer Protein type 5	-	At2g37870		Sequence similarity	0	S
Lipid Transfer Protein type 5	-	At2g44290		Sequence similarity	1	S
Lipid Transfer Protein type 5	-	At2g44300		Sequence similarity	2	S
Lipid Transfer Protein type 5	-	At2g48130		Sequence similarity	0	M
Lipid Transfer Protein type 5	-	At2g48140		Sequence similarity	0	_
Lipid Transfer Protein type 5	-	At3g22570		Sequence similarity	0	S
Lipid Transfer Protein type 5	-	At3g22580		Sequence similarity	1	S
Lipid Transfer Protein type 5	-	At3g22600		Sequence similarity	0	S
Lipid Transfer Protein type 5	-	At3g22620		Sequence similarity	0	S
Lipid Transfer Protein type 5	-	At3g43720		Sequence similarity	2	S
Lipid Transfer Protein type 5	-	At4g08670		Sequence similarity	1	S
Lipid Transfer Protein type 5	-	At4g12360		Sequence similarity	2	S
Lipid Transfer Protein type 5	-	At4g14815		Sequence similarity	1	S
Lipid Transfer Protein type 5	-	At4g22630		Sequence similarity	2	S
Lipid Transfer Protein type 5	-	At4g22640		Sequence similarity	1	S
Lipid Transfer Protein type 5	-	At5g09370		Sequence similarity	0	S
Lipid Transfer Protein type 5	-	At5g13900		Sequence similarity	0	S
Lipid Transfer Protein type 5	-	At5g64080		Sequence similarity	2	S
Lipid Transfer Protein type 6	-	At4g22490		Sequence similarity	0	S
Lipid Transfer Protein type 6	-	At4g22520		Sequence similarity	0	S
Lipid Transfer Protein type 7	-	At3g58550		Sequence similarity	0	S
Lipid Transfer Protein type 8	-	At4g28395	ATA7	Sequence similarity	0	-

Figure 1: Lipid diffusion inside and across lipid bilayers. The figure shows the half-times (T½) for spontaneous interbilayer and transbilayer movement according to [12, 16]. Large arrows indicate fast movement and dashed arrows indicate slower movement. P: phosphate, Chol: choline, Gal: galactose.

Figure 2: Lipid transfers towards and outwards the chloroplast envelope. PC is present in the outer leaflet of the outer envelope membrane but is synthesized in the ER, not in the plastid. It may be transferred from ER to the envelope directly or through its conversion to LysoPC. The main lipids of thylakoids i.e. galactolipids, PG and SQDG are formed in the envelope. Whereas prokaryotic type galactolipids are issued from phosphatidate (PA_P) synthesized in the chloroplast, formation of eukaryotic type galactolipids is dependent on the supply to the envelope of some PC derivatives formed in the ER: either diacylglycerol ($DAG_{E)}$ [6] or phosphatidate (PA_E) [37]. PAP converting PA to DAG is present in the envelope but only in the inner membrane. Altogether, a number of lipid transfers noted by dashed arrows are important to build plastid membranes. Under phosphate deprivation, DGDG formation is stimulated corresponding to activation or stimulation of a part of the galactolipid synthesis pathway indicated in red [42; 6; 9]. Under these conditions, DGDG is transferred through membrane contact between chloroplast and mitochondria [9].

Figure 3: Scheme of the plant secretory pathway according to [48]. This scheme represents different routes starting from the ER and indicates key proteins involved in vesicle trafficking. Phosphorylated forms of PI address vesicles to specific compartments: PI-4P to the storage vacuole, PI-3P to the lytic vacuole and PIP2 to the plasma membrane. 1- Transfer from ER to Golgi uses COPII type vesicle machinery. Identified proteins involved in this transfer are the GTPase Sar1, the GTP exchanging factor Sec12, the coat complexes Sec13 and Sec23, and the GTPase RabD. 2- Transfer from Golgi to ER uses a COPI type vesicle

machinery. Identified proteins involved in this transfer are the GTPase Arf1, interacting with a transmembrane protein p23, the GTP exchanging factor Gea, H/KDEL motif receptor responsible of protein retrograde traffic ERD2. This transfer is actin dependent. 3- Transfer from ER and Golgi to storage vacuole with the intervention of dense vesicles is not yet characterised. The only protein identified in this transfer is the storage vacuole addressing receptor PV72. 4- Transfer from Golgi to lytic vacuole *via* prevacuolar compartment uses clathrin vesicle machinery. Identified proteins involved in vesicle formation are the GTPase Arf1, lytic vacuole addressing motif receptor BP80 able to recruit the adaptatin complex AP1 and thus clathrin. Vesicle fusion to prevacuolar compartment involves the v-SNARE PEP12 and VAM3, the t-SNARE SYP25 and VTI11, and the GTPases RabA and RabF. This transfer is actin and dynamin ADL6 dependent. 5- Transfer from Golgi to plasma membrane is not yet identified. Protein involved in vesicle formation or fusion are not known. Only two PI-TP, Sec14 and Sfh1, were characterised. This transfer is actin dependent. 6- Endocytosis uses clathrin coated vesicle machinery. Identified proteins involved in vesicle formation are the adaptatin complex AP2 and clathrin. This transfer is actin and dynamin ADL1 dependent. And 7- Exocytosis utilizes a vesicle machinery not yet characterised. Identified proteins involved in vesicle formation are the GTPase Arf1 and the GTP exchanging factor GNOM. Vesicle fusion to plasma membrane involves the t-SNARE KNOLLE and the v-SNARE SNAP33.

Figure 4: Lipid transfer at membrane contact sites. A: Electron microscopy observation of *A. thaliana* cells grown in suspension. Cells were grown for 3 days in minus P_i medium. Bar: 1 µm. Arrows indicate position of contact between mitochondria and amylochloroplasts. B: Schematic representation of membrane contact sites reported in the plant cell. Reticulum

forms a network that facilitates lipid transfer between organelles. Plastids create envelope extension in the form of stromules or of myelinic structures, marked with a star.

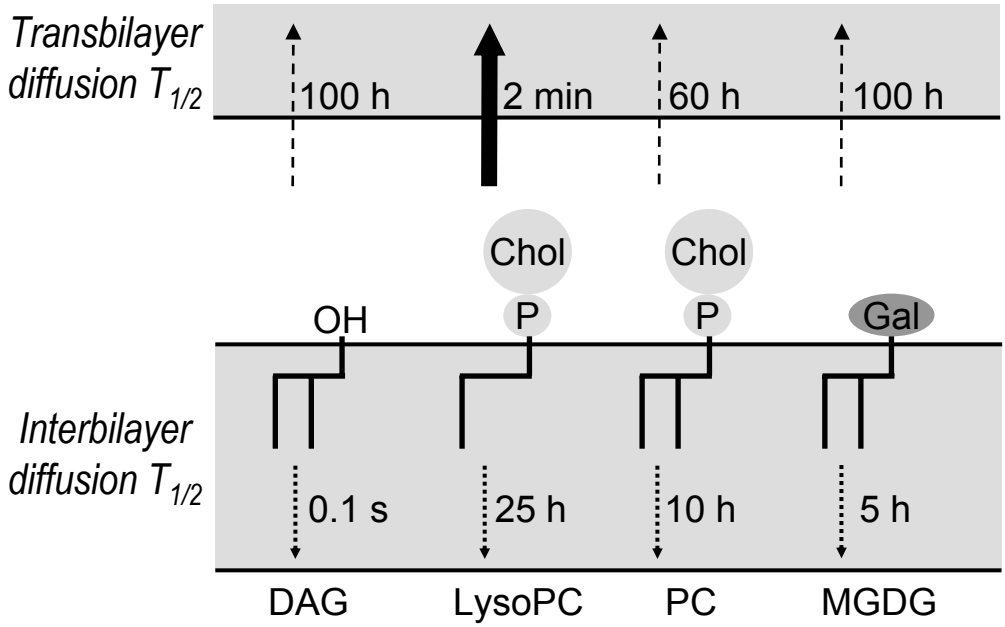
Transbilayer diffusion $T_{1/2}$
100 h
2 min
60 h
100 h
Chol
Chol
OH
P
P
Gal
Interbilayer diffusion $T_{1/2}$
0.1 s
25 h
10 h
5 h
DAG
LysoPC
PC
MGDG

Figure 1

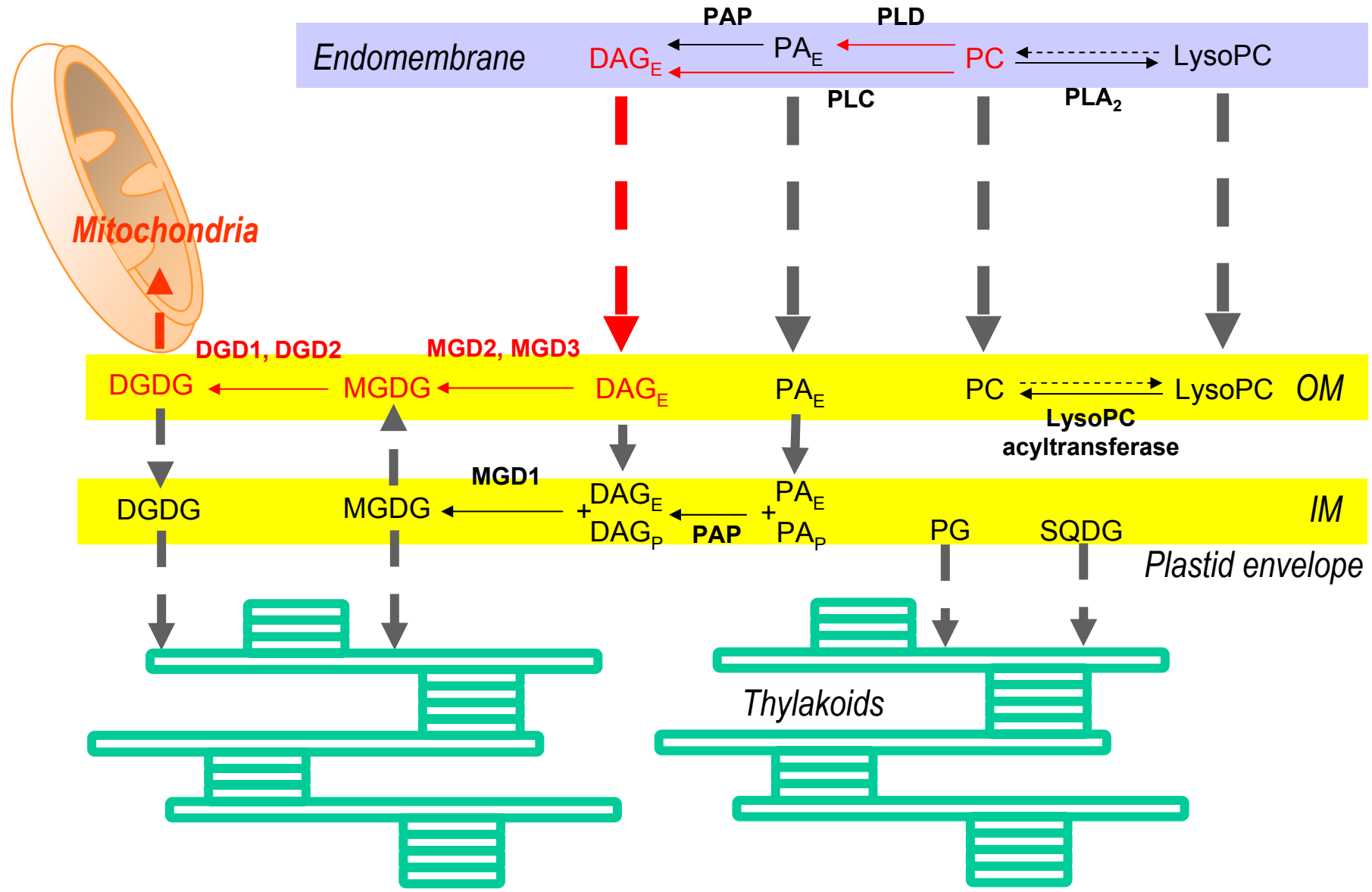
Endomembrane
PAP
PLD
DAG$_E$
PA$_E$
PC
LysoPC
PLC
PLA$_2$
Mitochondria
DGD1, DGD2
MGD2, MGD3
DGDG
MGDG
DAG$_E$
PA$_E$
PC
LysoPC
OM
LysoPC
acyltransferase
MGD1
DGDG
MGDG
DAG$_E$
+
DAG$_P$
PAP
PA$_E$
+
PA$_P$
PG
SQDG
IM
Plastid envelope
Thylakoids

Figure 2

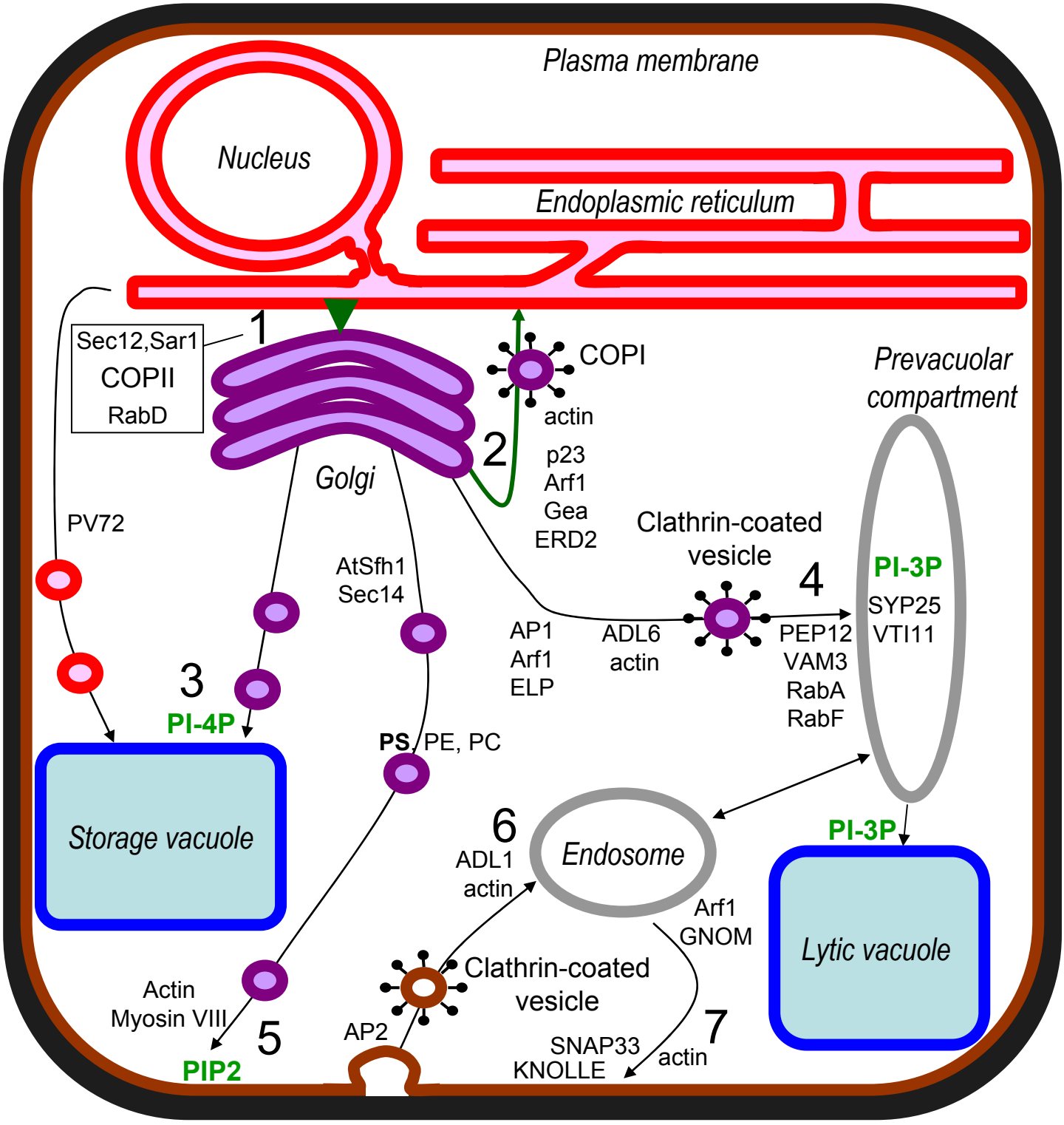
Plasma membrane
Nucleus
Endoplasmic reticulum
Sec12,Sar1
COPII
RabD
1
COPI
actin
2
p23
Arf1
Gea
ERD2
Golgi
Prevacuolar
compartment
PV72
AtSfh1
Sec14
Clathrin-coated
vesicle
4
PI-3P
SYP25
VTI11
AP1
Arf1
ELP
ADL6
actin
PEP12
VAM3
RabA
RabF
3
PI-4P
PS, PE, PC
Storage vacuole
6
ADL1
actin
Endosome
PI-3P
Arf1
GNOM
Lytic vacuole
Actin
Myosin VIII
5
AP2
Clathrin-coated
vesicle
7
SNAP33
KNOLLE
actin
PIP2

Figure 3

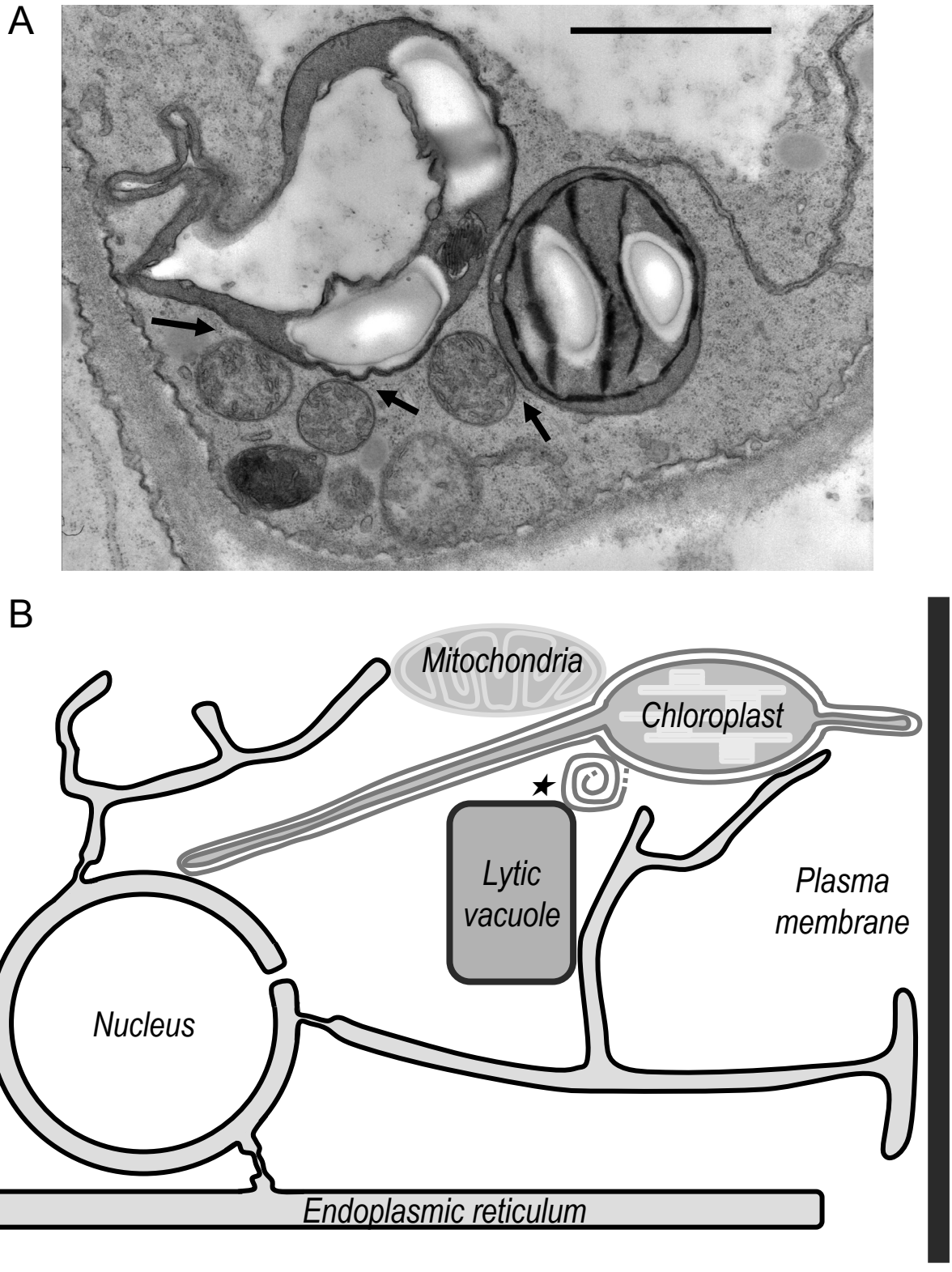
A
B
Mitochondria
Chloroplast
Lytic vacuole
Plasma membrane
Nucleus
Endoplasmic reticulum

Figure 4